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TECHNICAL MANUSCRIPT 276

DISTRIBUTION OF LYMPHATIC TISSUE AND β-GALACTOSIDASE-POSITIVE CELLS IN THE SPLEEN OF NEW ZEALAND RABBITS

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DIRECTORATE OF MEDICAL RESEARCH

Project 1L013001A91A

January 1966

In conducting the research reported here, the investigators adhered to "Principles of Laboratory Animal Care" as established by the National Society for Medical Research.

ABSTRACT

New Zealand rabbits were injected with 7S human γ-globulin, fraction II human γ-globulin, and isopropyl thiogalactoside and sac ified at 4, 24, 48, 72, 96 hours, and 7 days. Marginal and central zones of lymphoid follicles measured 0.15227 ± 0.012 and 0.08527 ± 0.004 mm^2. The 7S γ-globulin showed changes in marginal zone but only in 48 hours and 7 days in the central zone. Fraction II showed no changes. The number of polymorphonuclear leukocytes with 7S γ-globulin showed marked increase but none with isopropyl-β-D-thiogalactopyranoside. There were no changes in reticular cells known to be β-galactosidase-positive. The 5-bromo-4 chloroindol-3-yl-β-D-galactopyranoside was used to demonstrate enzyme. Changes were instituted in substrate that enabled us to study enzyme in 2-μ sections at short incubation time, increasing the sensitivity of the reaction. The enzyme was distributed in reticular cells around central zone, trabeculae, and cortical sinuses. Enzyme changes apparently were not associated with cellular changes.

DISTRIBUTION OF LYMPHATIC TISSUE AND β-GALACTOSIDASE-POSITIVE CELLS IN THE SPLEEN OF NEW ZEALAND RABBITS

Previous studies have shown the presence of β-galactosidase-positive cells in the spleen of rats.* Other studies have also shown increased β-galactosidase-positive cells in the rat spleen following the intravenous injection of isopropyl-β-D-thiogalactopyranoside. No changes occurred following injection of bovine γ- lobulin. These studies were made only during and up to the first hour after the injection. Thick frozen sections (6 to 8 μ) with long incubation times in the substrate were used to demonstrate the enzyme.**

It was thought that more definitive results could be found by studying the early stages of cellular changes occasioned by injection of γ-globulins and isopropyl-β-D-thiogalactopyranoside. The rabbit was used because the ease of perfusion of the spleen afforded a better insight into the distribution of lymphatic tissue following the injection of the above substances. Also, no demonstration of β-galactosidase histochemically has been shown in the rabbit. In addition, the added possibility of enzyme diffusion with thick sections (6 to 8 μ) and relative long incubation times made it necessary to make pertinent changes in our substrate conditions to reflect a higher degree of enzyme sensitivity and specificity.

New Zealand rabbits were used for this study. At necropsy the splenic vessels were cannulized and perfused with Tyrode's solution to permit more accurate localization of the distribution of lymphatic tissue. The areas of the marginal and central zones of the lymphoid follicles were measured in mm^2 and are tabulated in Table 1. Area measurements in the control group were: marginal zone 0.15227 ± 0.012 and central zone 0.08527 ± 0.004. Following injection of 10 mg 7S human γ-globulin there were significant changes of size of the marginal zone after 4, 24, 48, 72, 96 hours, and 7 days. This consisted of a gradual decrease in this area during each time interval until 48 hours, when the area measurement was 0.12674 ± 0.008 mm^2, and a gradual increase in the area measurement reaching 0.17564 ± 0.019 at 7 days. Injection of 10 mg of Cohn's human fraction II γ-globulin showed no significant changes in either zone during the same period.

The numbers of polymorphonuclear leukocytes adjacent to the central zone were studied relative to the unit areas of 0.0009 mm^2 (Table 2). In groups injected with 10 mg 7S human γ-globulin there was a definite increase

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* Pearson, B.; Wolf, P.; Vazquez, J. 1953. A comparative study of a series of new indolyl compounds to localize β-galactosidase in tissue. Lab. Invest. 12:1249-1259.

** Pearson, B.; Wolf, P. 1964. Increased β-galactosidase synthesis in lymphatic system following injections of isopropyl-β-D-thiogalacto-pyranoside. Federation Proc. 23:549.

TABLE 1. SIZE OF SPLENIC LYMPHOID FOLLICLES IN NEW ZEALAND RABBITS

Hours	Marginal Zone, a/ Area, mm^2	pc/	Central Zone, a/ Area, mm^2	pc/	Marginal Zone, b/ Area, mm^2	pc/	Central Zone, b/ Area, mm^2	pc/
0	0.15227 ± 0.012		0.08527 ± 0.004		0.15227 ± 0.091		0.08527 ± 0.0001	
4	0.13505 ± 0.018	0.05	0.07403 ± 0.010	0.5	0.16091 ± 0.012	0.9	0.08786 ± 0.0050	0.9
24	0.12263 ± 0.014	0.001	0.06651 ± 0.008	0.7	0.15063 ± 0.011	0.9	0.08387 ± 0.0070	0.9
48	0.12674 ± 0.008	0.001	0.08344 ± 0.007	0.9	0.12737 ± 0.022	0.5	0.07455 ± 0.0090	0.5
72	0.17034 ± 0.014	0.01	0.11508 ± 0.011	0.05	0.14828 ± 0.016	0.9	0.07700 ± 0.0110	0.5
96	0.16557 ± 0.014	0.05	0.10765 ± 0.009	0.05	0.17795 ± 0.027	0.5	0.08405 ± 0.0060	0.9
7 days	0.17564 ± 0.019	0.01	0.10793 ± 0.010	0.1	0.14623 ± 0.017	0.9	0.08632 ± 0.0120	0.9

a. 10 mg 7S human γ-globulin injected subcutaneously.
b. 10 mg Cohn's human fraction II injected subcutaneously.
c. Probability from Fisher's tables; underscored values are significant.

TABLE 2. POLYMORPHONUCLEAR LEUKOCYTES PER 0.0009 mm^2 IN LYMPHOID FOLLICLES ADJACENT TO CENTRAL ZONE

Hours	10 mg 7S Human γ-Globulin Injected		10 mg Isopropyl-β-D-Thiogalactopyranoside Injected Subcutaneously	
	Number	p[a]	Number	p[a]
0	0.1094 ± 0.0267		0.1094 ± 0.0267	
4	0.7639 ± 0.3201	0.001	0.2083 ± 0.1249	0.3
24	0.5972 ± 0.1420	0.001	0.1250 ± 0.0796	0.9
48	0.5278 ± 0.0796	0.001	0.1250 ± 0.0832	0.9
72	0.3333 ± 0.0804	0.001	0.1805 ± 0.0453	0.3
96	0.2500 ± 0.0120	0.1	0.1805 ± 0.0102	0.5
7 days	0.1667 ± 0.0861	0.5	0.1805 ± 0.1164	0.5

a. Probability from Fisher's tables; underscored values are significant.

in the numbers of polymorphonuclears at 4, 24, 48, and 72 hours. This was greatest at the 4-hour measurement, with gradual diminution until after the 72-hour measurement. Injection of isopropyl-β-D-thiogalactopyranoside showed no decreases or increases in polymorphonuclears during the periods inclusive of zero through 7 days.

Because β-galactosidase histochemistry specifically is involved with reticular cells and sinus reticulum cells (exclusive of primitive reticulum cells) the spleens of animals subjected to injection of the previously mentioned materials were studied. No apparent maldistribution or qualitative and semi-quantitative changes could be discerned. The only change that could be readily seen was the increase in polymorphonuclear leukocytes that penetrated and infiltrated the red pulp and extended into the marginal zone adjacent to the central zone.

Previous studies* indicated some 700% increase in galactosidase-positive cells following injection of isopropyl thiogalactoside, but no increase following the injection of bovine γ-globulin. In our present material there was no maldistribution of reticular or sinus reticulum cells, nor any increases following injection of 7S γ-globulin nor isopropyl thiogalactoside. The only increase was in the number of polymorphonuclear leukocytes following injection of the former but not the latter material. Furthermore, polymorphonuclear leukocytes do not show β-galactosidase activity relative to the condition of our experiments.

The β-galactosidase histochemistry was studied in frozen sections of rabbit spleen sliced to 2 μ in thickness in a Harris cryostat at -20 C. They were incubated from 3 to 4 hours in a substrate containing 2.0 mg 5-bromo-4 chloroindol-3-yl-β-D-galactopyranoside in 0.5 ml dimethyl formamide, 0.5 ml of 0.85% NaCl; 2.0 ml of 0.2 M acetate buffer (pH 5 to 4); and 8 mg spermidine hydrochloride and 15 ml distilled water. The reaction can be seen at 30 minutes and continues to intensify at 1, 2, and 3 hours reaching a maximum at 4 hours.

The substrate 5-bromo-4 chloroindol-3-yl-β-D-galactopyranoside is specifically hydrolyzed by β-galactosidase in tissue to form a 5,5'-bromo-4,4'-chloroindigo that is localized to enzymic sites (Figure 1). It is highly substantive to protein and does not diffuse, giving a sharp intense color to cells containing the enzyme. Under our present modified method the sensitivity of the reaction is extremely high so that it can be readily studied in 2-μ sections with relative short incubation times, eliminating traces of artifact due to enzyme diffusion.

The distribution of the enzyme shows a characteristic arrangement around and adjacent to the central zone of the follicles (Figure 2), around the trabeculations of the spleen (Figure 3) and in the cortical sinuses (Figure 4). The cells involved are those of fixed reticular cells in the sinuses. However, not all cells of this type react, as none are present in the central zone where reticular cells may be abundantly present. Only about 1 to 2% of the reticular cells react. Stimulated reticular cells containing ingested material seem to have a higher degree of reaction than nonstimulated cells. This is in line with Dannenberg and Dennett's studies of free cells of the reticuloendothelial system, which shows a higher degree of activity (esterases and phosphatases).**

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* Pearson, B.; Wolf, P.; Vazquez, J. 1963. A comparative study of a series of new indolyl compounds to localize β-galactosidase in tissue. Lab. Invest. 12:1249-1259.
Pearson, B.; Wolf, P. 1964. Increased β-galactosidase synthesis in lymphatic system following injections of isopropyl-β-D-thiogalactopyranoside. Federation Proc. 23:549.

** Dannenberg, A.M., Jr.; Dennett, W.E. 1963. Hydrolases of mononuclear exudate cells and tuberculosis: I. Exudate characteristics, esterases, proteinases, and tissues. Arch. Pathol. 76:589-591.

The principle of the reaction for β-Galactosidase

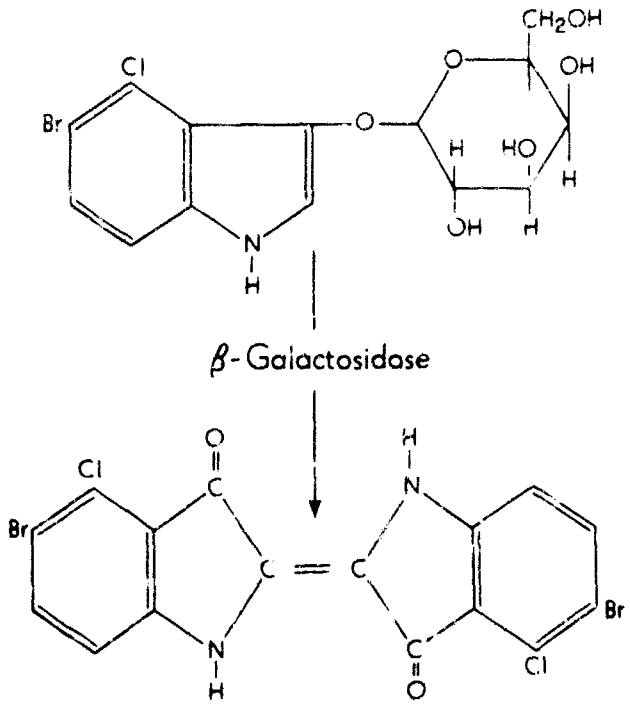

Figure 1. Principle of the Reaction for Demonstrating β-Galactosidase in Tissue Sections. The soluble colorless 5-bromo-4 chloroindol-3-yl-β-D-galactopyranoside is hydrolyzed by the β-galactosidase in tissue to form the highly chromogenic insoluble final product 5,5'-bromo-4,4'-chloroindigo at enzymic sites.

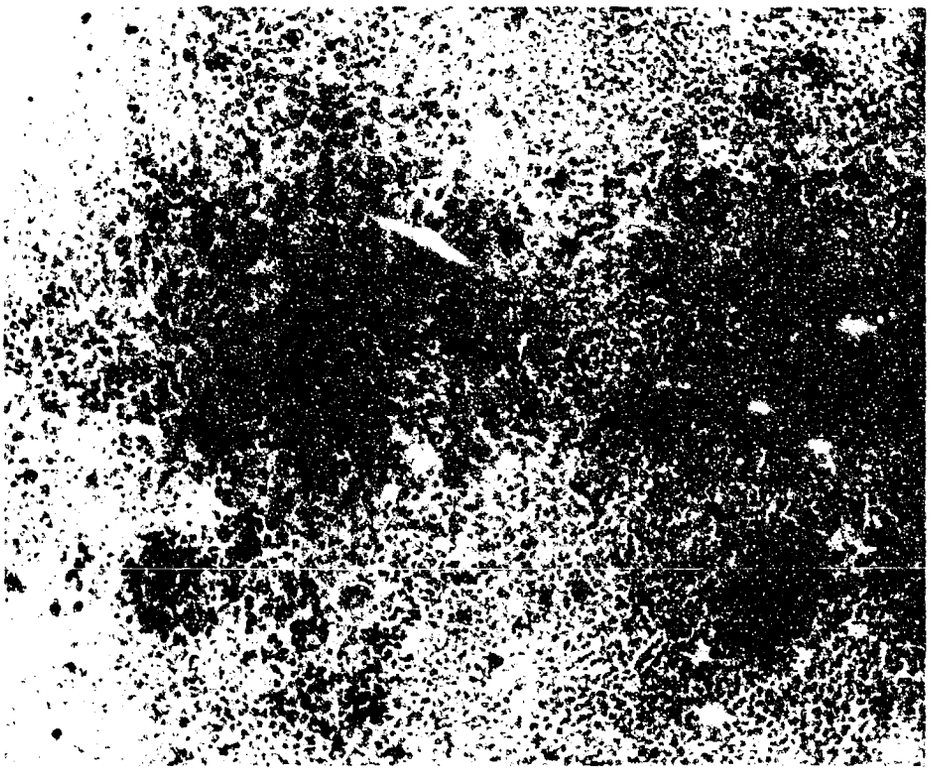

Figure 2. β-Galactosidase Positive Cells Shown in Spleen Around Lymphoid Follicle. 200X

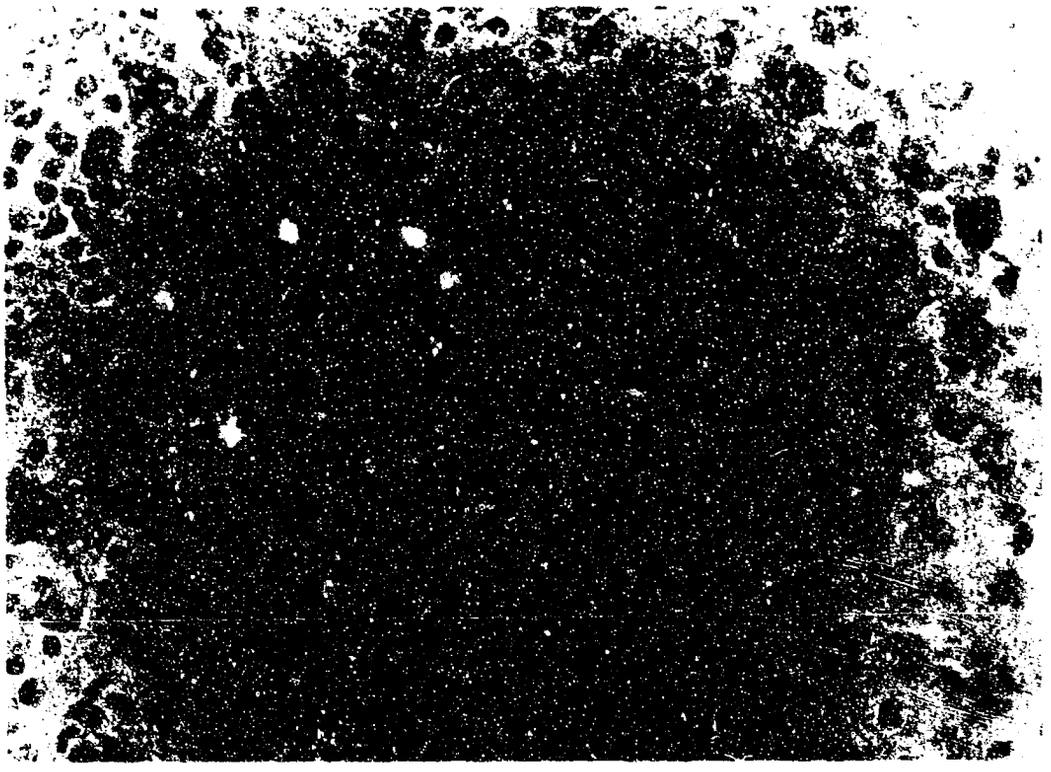

Figure 3. β-Galactoside Positive Cells' Shown Adjacent to Trabeculation of the Spleen. 800X

Figure 4. β-Galactosidase Positive Cells' Shown in the Sinus Reticulum Cells. 200X

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